SUPPLEMENTAL DATA– Lim et al.

Supplemental Figure 1: Characterization of myosin II-inhibited wound edge cells

(A) Immunolocalization of F-actin, myosin-II and adhesions in blebbistatin-treated cells. Left panel: Phalloidin staining of F-actin distribution in treated wound edge cell. Note the absence of dense F-actin bundles. Scale bar: 10 µm. Right panels: F-actin staining (red), myosin II staining (green), and paxillin staining (cyan) indicate diffuse organization of cytoskeleton elements. Tiny adhesions (arrowheads) at the base of the lamellipodia are sufficient to balance the propulsive forces induced by F-actin assembly at the cell edge. **(B)** Left panel: Edge evolution of blebbistatin-treated cell. Scale bar = 10 µm. Mid panel: Instantaneous F-actin flow. Lamellar retrograde flow is decreased, but lamellipodia flow is unchanged [1]. Right panel: F-actin speed averaged over 100 s. Units are in µm/min.

Video Legend

Video 1: Formation of Leader cells. Time-lapse phase images of leader cell formation. Images were acquired for every 2.5 minutes, for total observation period of 3 hours (replay movie sped up 3600X). Follower cells are seen flanking leaders cells on either side.

Video 2: Edge tracking of leader (left panel), follower (mid panel) and island cells (right panel). Top row displays edge evolution. Bottom row shows edge tracking (red border lines) of X-rhodamine-actin microinjected cells (speckles). Images were acquired at 10 second time intervals for 8 minutes (replay movie sped up 125X).

Video 3: qFSM analysis of F-actin dynamics in leader cell. Left panel: Leader cells co-microinjected with eGFP-mRLC (green) and X-rhodamine conjugated actin (red). Images were acquired every 10s for 8 minutes (replay movie sped up 125X). Mid panel: F-actin flow maps

(yellow vectors) generated from actin speckles. Right panel: zoomed region of contraction zone as depicted in the mid panel. F-actin retrograde (green squares) and anterograde (red squares) flows overlap and interdigitate in leader cells (Interpenetrating-contraction mode). Only speckles with trajectories lasting longer than 5 frames (50s) are shown (red or green squares).

Video 4: qFSM analysis of F-actin dynamics in island cell. Left panel: Island cell co-microinjected eGFP-mRLC (green) and X-rhodamine conjugated actin (red). Images were acquired every 10s for 8 minutes (replay movie sped up 165X). Edge retraction leads to formation and actomyosin transverse arcs (bundling-contraction mode). Right panel: F-actin flow. Note the fast retrograde flow anterior of the transverse arc in comparison to the slow anterograde flow posterior to the transverse arc.

[1] Ponti, A., Machacek, M., Gupton, S.L., Waterman-Storer, C.M., and Danuser, G. (2004). Two distinct actin networks drive the protrusion of migrating cells. Science *305*, 1782-1786.